Pedobiologia 39, 207–218 (1995)
Gustav Fischer Verlag Jena

Dispersal and fragmentation of the enchytraeid *Cognettia sphagnetorum* in metal polluted soil

M. Sjögren*, A. Augustsson and S. Rundgren

Department of Ecology, Lund University, S-22362 Lund, Sweden

Summary. A number of patches of high quality will most probably be present even in soil of a generally low quality, e.g. caused by a patchy distribution of industrially emitted metal pollution, making it possible for soil invertebrate populations to sustain at low densities. The abilities to disperse, recognize a favourable patch, orientate towards it and successfully colonize it will then increase the survival potential of an individual and its progeny. To understand how enchytraeids can survive in metal polluted soil a number of laboratory experiments were carried out. They focussed on dispersal ability, reproduction and the ability to avoid polluted soil of the enchytraeid worm *Cognettia sphagnetorum* (Vejdovsky). The worm reproduces by dividing into two to several fragments which regenerate head, tail or both. Adults (>35 segments) of *C. sphagnetorum* sampled in mor soil of a coniferous forest were used to test the impact of metal polluted (mainly Cu + Zn) soil on the variables mentioned above. *C. sphagnetorum* had a rather low dispersal rate which was unaffected by soil metal concentrations; 5% of the population covered 6 cm within two weeks while 75% did not move at all. The species avoided high metal concentrations and, when forced to inhabit metal polluted soil, the mortality rate increased in soils with more than 1670 mg Cu + Zn kg^{-1} dry mass. The highest rate of fragmentation was found in soil with a metal content of 3300 mg Cu + Zn kg^{-1} soil dry mass and more fragments than unchanged adults were found in soils containing more than 850 mg Cu + Zn kg^{-1} soil dry mass, suggesting an induced fragmentation. There seemed to be an upper limit to the accumulation of copper (20 µg Cu mg^{-1} dry mass body tissue), while zinc was accumulated in direct proportion to the concentration of the substrate. *C. sphagnetorum* is a potential candidate for use in soil toxicity tests focussing on biological variables as it is abundant and inhabits the uppermost centimeters of the soil layer which often is most affected by anthropogenic activities.

Key words: Enchytraeidae, copper, zinc, dispersal, fragmentation, toxicity testing

Introduction

A heterogeneous distribution of food resources has been recognized in models on the impact of dispersal on the distribution of above-ground insect populations (Kareiva 1982; 1983; Turchin 1991). In these studies the patches were easily definable as individual or groups of plants. In the root matrix, however, the patch is still hard to define, but a number of examples are given in the literature. For instance, the succession of micro-organisms on pieces of newly fallen wood (Swift & Boddy 1984), the concentration of exudates around a root (Coleman et al. 1984), and root-mycorrhiza associations (Frankland 1992) will affect

Correspondence to: M. Sjögren

the presence and distribution of animals feeding upon micro-organisms or depending on nutrients exudated. The spatial variation in food abundance is associated with a heterogeneity of physical and chemical properties (humidity, temperature, porosity, thickness of the humus layer etc). On a micro-scale the soil environment can thus be visualized as a dynamic system of patches or microhabitats, varying in quality, i.e. suitability to a given organism.

Soil animals may move between favourable patches where they stop for feeding, reproducing etc. either randomly or guided by physical (e.g. soil humidity (Usher 1976)) or biotic gradients (e. g. odour plumes (Bengtsson et al. 1988b, 1991). To maintain a population in such a spatial system, soil invertebrates could benefit from a high dispersal ability and asexual reproduction. When a high quality patch is degraded the individual is forced to find another adequate patch to exploit. The abilities to move, recognize and orientate towards such a patch are then essential. Being asexual increases the chances to successfully colonize the new patch as it leads to a doubling of fecundity, although the advantage of genetic recombination is lost (Maynard Smith 1971).

Even a soil of a generally low quality to a given organism, for instance due to metal pollution, contains patches of acceptable quality (low metal concentrations) as indicated by the high variation in metal concentrations in small samples of soil from contaminated sites (van Capelleveen et al. 1986; Bengtsson & Rundgren 1988). The heterogeneous distribution of metals together with the high variation in hyphal density found in polluted soil (Nordgren et al. 1983) and the shift in the fungal community towards metal tolerant fungal species (Arnebrant et al. 1987) indicate the existence of patches of relatively good quality even in a severely polluted soil. Such patches may make it possible for an invertebrate population to sustain, though at low densities, as shown for enchytraeids in metal polluted soils around a brass mill (Bengtsson & Rundgren 1982). Alternative mechanisms for maintaining a population in a polluted environment would be adaptations involving detoxification or shifts in feeding habits and continuous immigration waves.

This study aimed at assessing: 1) the dispersal ability of the enchytraeid *Cognettia sphagnetorum* (Vejdovsky) in natural soil and in a soil of lowered quality caused by industrially emitted metals; 2) the species avoidance of low quality soil patches; 3) the impact of enhanced soil metal concentrations on fragmentation, and thus the population growth of *C. sphagnetorum*; 4) the uptake of copper and zinc from the soil substrate and the accumulation of metals in the enchytraeid body.

Materials and Methods

Test organism. In most temperate soils enchytraeid worms occur at high densities; 50000 – 300000 ind. m^{-2} have been recorded (Peachey 1963; Springett 1970; Abrahamsen 1972). In Fennoscandian coniferous forest soils *C. sphagnetorum* is the dominating enchytraeid species (Abrahamsen 1972; Lundkvist 1982; Bengtsson & Rundgren 1982; Huhta 1984). It is a 10 – 15 mm long, thin and white worm that chiefly seems to reproduce asexually by fragmentation (Christensen 1959) resulting in the production of head, tail, and sometimes middle fragments (Standen 1973). In 28 days at 10 °C tail fragments (Springett 1970) and in 14 days at 15 °C (A. Augustsson & S. Rundgren unpubl.) tail and middle fragments have regenerated a functional mouth and can start feeding. After another two months the regenerating fragment is indistinguishable from a whole worm (Springett 1970).

Enchytraeids respond to changes in soil humidity by migrating vertically through the soil profile both in the field (Nielsen 1955; Peachey 1963) and in controlled laboratory experiments (Dash & Cragg 1972). Diel vertical migrations in the uppermost 6 – 10 cm of the soil in response to humidity (Springett et al. 1970) and temperature and oxygen saturation (Erman 1973) have also been confirmed. The ability of enchytraeids to form their own burrows is limited (Didden 1990), and they are therefore mostly confined to move in already existing cavities and crevices in the soil (Wallwork 1976).

All specimens of *C. sphagnetorum* used in the experiments were extracted from mor soil collected at the reference site (see below) using the wet funnel extraction method (O'Connor 1967). They were

determined under light-microscope (× 10) and were immediately transferred with a needle to the experimental rings (see below) to avoid handling that could impose stress and cause injuries. Only unhurt and healthy looking specimens >35 segments (head segment excluded) were used in the experiments. Like Makulec (1983), we consider *C. sphagnetorum* of that size to be adult, i.e. able to reproduce by fragmentation.

Experimental soils. The experiments were carried out using mor soils collected in coniferous forests (*Vaccinium* biotope) in the vicinity of the village of Gusum, SE Sweden, where a brass mill has been in operation since 1661. In 1966 the old mill was replaced by a new one erected 1.5 km W of the village centre. Zinc and copper form about 98% of the metal emission from the new mill (Tyler 1974).

Mor soils were sampled at two sites: a heavily metal polluted site located 400 m NW of the new mill in the prevailing wind direction (site M1 in Tranvik et al. 1994) and a reference site 7800 m further NW (site V in Bengtsson & Rundgren 1982). The soils were sieved (mesh size 5.9 mm) to remove large particles and then frozen and thawed three times to kill indigenous fauna.

To estimate water holding capacity, the two experimental soils were loosely packed in plastic cylinders (20 mm h, 36 mm diam.) (n = 5; reference soil and n = 4; polluted soil) and incubated for 48 h at 5 °C to settle. The cylinders were water saturated in a tray with 1 cm water for 24 h and then allowed to drain for another 24 h. The samples were weighed and then dried at 105 °C to constant weight. Water holding capacity (here corresponding to approx. pF 0.5; see Abrahamsen 1971) was estimated from the differences in weight between the drained and dried soil sample. The soils of the two sites differed in water holding capacity (Table 1), presumably owing to the impact of metals on soil structure in the polluted site. Maximum growth of *C. sphagnetorum* populations in raw humus (mor) has been found to occur when water content corresponds to 50–95% of water holding capacity (Abrahamsen 1971). Soil moisture of the reference soil was therefore set to 60% fresh mass, which corresponds to 73% of water holding capacity. To obtain equal moisture conditions of the polluted soil in % of water holding capacity, the water content of this soil was adjusted by adding distilled water to 42% fresh mass.

Table 1. Total concentrations of copper and zinc (n = 2) in a series of experimental soils obtained by mixing soil sampled at two coniferous forest sites in an area affected by a brass mill near the village of Gusum, SE Sweden. Mycelial length (n = 3) and water holding capacity (n = 4) (% of water saturated weight) are given for some of the experimental soils. The reference site and the heavily polluted site are described in Bengtsson & Rundgren (1982) and Tranvik et al. (1994). Mean and SD are given

experimental soils reference : polluted	metal concentrations (mg kg^{-1} dry mass)		mycelial length (m g^{-1} dry mass)	water holding capacity (%)
	Cu	Zn		
1 : –	14 ± 1	116 ± 1	191 ± 111	82 ± 1
15 : 1	226 ± 26	434 ± 4		
7 : 1	300 ± 1	550 ± 34		
3 : 1	590 ± 49	1080 ± 42	160 ± 62	
1 : 1	1468 ± 41	1832 ± 140		
– : 1	2648 ± 33	4182 ± 502	18 ± 10	57 ± 2

Soil from the reference site was added to the heavily polluted soil so that metal concentrations were stepwise halved giving rise to four mixed experimental soils in addition to the field collected ones (Table 1). Soil metal (henceforth = Cu + Zn) concentrations were analysed. Duplicate soil samples (approx. 2 g) were dried at 105 °C for 24 h. Each sample was heat digested in concentrated nitric acid and perchloric acid (4 : 1 vol : vol). Metal concentrations were determined by flame atomic absorption spectrophotometry (Varian SpectrAA 300/400 Zeeman).

Potential food (O'Connor 1967; Brockmeyer et al. 1990) was expressed as metabolically active mycelial length. Estimates of hyphal lengths were made on three sub-samples of three of the experimental soils, viz. 130, 1670, 6830 mg Cu + Zn kg^{-1} soil dry mass, with the FDA staining method (Söderström 1977). Freezing and thawing the experimental soils seems to have only minor negative effects on the amount of fungal mycelium (Bengtsson et al. 1988a).

Dispersal. PVC-rings (20 mm h, 36 mm diam.) were half-filled with either reference soil (130 mg Cu + Zn kg^{-1} soil dry mass) or soil of 1670 mg Cu + Zn kg^{-1} soil dry mass (see Table 1). Thirty adult specimens of *C. sphagnetorum* were placed on the soil surface of a ring. When evident that all specimens were unhurt, the corresponding soil was added until the ring was completely filled. It was sealed with nets (mesh size 60 µm) at both ends to permit air exchange, and left overnight at 15 °C in darkness to allow the worms to acclimatize. On the following day the nets were removed and the worm-containing ring was connected with adhesive tape to three already joined worm-free rings filled with the corresponding soil. The four rings thus made up a tube of 80 mm in length with the worm-containing ring at one end. The tubes (n = 6) were sealed with nets at both ends as above and incubated horizontally in darkness at 15 °C for two weeks. To reduce water losses they were kept in plastic cases (115 × 240 mm) with a moist bottom layer of plaster of Paris and plastic lid. Evaporated water was compensated for by adding distilled water to the tubes once or twice in the course of the experiment. At the end of the experiment the rings were separated and the enchytraeids were separately extracted using the wet funnel extraction method (O'Connor 1967). The worms were examined under light-microscope (× 10). The numbers of unfragmented worms and fragments (head, middle and tail) were determined and their body segments were counted.

Avoidance. To determine if *C. sphagnetorum* avoids a metal polluted soil, an experiment was carried out in which the worms could choose to stay in the reference soil or to move into the polluted soil. Thirty adult specimens were placed on the soil surface of a PVC-ring (20 mm h, 36 mm diam.) half-filled with reference soil. The ring was filled up with reference soil, sealed with nets (see above) and stored at 15 °C overnight to acclimatize the worms. On the following day the ring was joined to a second one containing one of the four experimental soils accounted for in Table 1. Six replicates of each of the following combinations of paired rings were run: 130 *vs* 130, 130 *vs* 660, 130 *vs* 850, and 130 *vs* 1670 mg (Cu + Zn kg^{-1} soil dry mass. The joined rings were incubated horizontally in plastic cases (see above) in darkness at 15 °C for four weeks. When the experiment was terminated the rings were split and the enchytraeids separately extracted. Worms obtained were examined as previously described.

It could be argued that the experimental setup would cause the metals to become evenly distributed in the two soils. However, it is known that metals are strongly bound to organic material and clay particles of natural soils (Lagerwerff & Specht 1970; Cavallaro & Mc Bride 1980). Hence, Tyler (1978) estimated that approximately 10% of the copper and lead in a heavily contaminated organic soil will leach within 80–120 and >200 years, respectively, whereas 10% of zinc and cadmium (metals that are more mobile) will leach within 9 and 20 years, respectively. In the field sites of the Gusum area there are considerable horizontal and vertical variations in absolute concentrations of copper, zinc, lead and cadmium (Bengtsson & Rundgren 1988; Bengtsson et al. 1992), which implies a low mobility of these metals in the experimental mor soils that were used in the present experiment. Had the study been based on artificial soils and a metal-salt solution added, the possibility to get a more even distribution of the metals over the rings would have been obvious.

Fragmentation. 30 ml of one of the six experimental soils (Table 1) was put in a plastic jar (40 mm h, 60 mm diam.). Ten healthy adult specimens were placed on the soil surface, and the jar was then sealed with plastic film provided with small holes to permit air exchange. The jars were placed in plastic containers (210 × 210 mm) with a plastic lid and a moist bottom layer of plaster of Paris and incubated in darkness at 15 °C for two weeks. At the end of the experiment the worms were extracted and examined as previously described. Seven replicates of each experimental soil were run.

Metal uptake. To document metal contents of exposed enchytraeids, healthy specimens were collected at the end of the fragmentation experiment. They were put in Ringer solution (7.5 g NaCl, 0.35 g KCl, 0.29 g $CaCl_2$ dissolved in 1000 ml deionized, distilled water) for four days to void gut contents, dried at 85 °C for 48 h in batches of 1–4 specimens, weighed using a Cahn Model 25 automatic electrobalance (precision ±0.1 µg), and put in 250 µl test tubes. 200 µl analytical grade nitric acid was added and the samples were heated (75 °C) until dry using an electrical heating block (Multi-block heater No. 2091). Three blanks were run parallel to each sample set to compensate for contaminants of reagents and test tubes. The samples (7–11 batches of worms reared in each soil type) were stored in a refrigerator until analysis. They were dissolved in 200 µl 0.1 M HCl one hour before analysis and the tubes capped with parafilm were turned upside down seven times. Metal concentrations were measured by atomic absorption spectrophotometry using a graphite furnace (Varian SpectrAA 300/400 Zeeman) (Bengtsson & Gunnarsson 1984).

Model of dispersal. To examine the distribution resulting from the dispersal experiment and find an appropriate model to predict and compare enchytraeid dispersal in differently polluted soils, observed and theoretical distributions were compared. The theoretical distribution was derived from a geometric model proposed by Buechner (1987). The model is based on the assumptions that a habitat is divisible

into discrete units each equally capable of supporting residents, and that dispersers have a constant probability to stop in any unit when passing. The formula used describes the density distribution of a population as the variable F(x).

$F(x) = p(1 - p)^x$, $x = 0, 1, 2, 3, \ldots$, where

x = the number of units passed by the individual before stopping and p = the probability to stop while crossing any one unit of the habitat. The p-value giving the best fit between observed and theoretical distributions was estimated using the Kolmogorov-Smirnov test (Sokal & Rohlf 1980).

Fragmentation rate. Two models to estimate fragmentation rate have been proposed. Standen's (1973) model was based on the determination of the number of worms at the start of an experiment and the number of unchanged specimens at the end (the experiments lasted 15–50 days). The differences found were ascribed to fragmentation and mortality. Makulec (1983) estimated the fragmentation rate from the numbers of head fragments and unfragmented worms at sampling (i.e. at the end of the experiment). The rate of fragmentation was then calculated using the formula:

$F = N_p/[d(N_z + N_p)]$, where

F = the proportion of worms that undergo fragmentation per day;
N_p = the number of regenerating head fragments at the end of the experiment;
N_z = the number of unfragmented (henceforth: unchanged, i.e. inoculated adult worms >35 segments, not fragmenting in the course of the experiment) worms at the end of the experiment;
d = experimental time (the time needed to producxe N_p).

Though we are well aware of the risk that head fragments may be lost due to the extraction efficiency and that there may be some mortality of inoculated worms, we use Makulec's model, since the experimental time was limited to two weeks.

Minimum population size. There are obvious problems to define mortality and calculate mortality rate in a fragmenting population (see e.g. Standen's (1973) predictive model). Due to the extraction method, fragments may remain in the soil, especially so middle and tail fragments that often are less mobile than head fragments. In the present study we therefore use the concept 'mimimum population size' when discussing fragmentation and survival of *C. sphagnetorum* in the experiments. Minimum population size is here defined as the sum of unchanged worms and all fragments extracted from a sample at the end of the experiment.

Results

Dispersal. At the end of the experiment more adult worms were recovered from the reference (81%) than from the metal polluted soil (57%) (p = 0.0038; Mann-Whitney U-test), whereas there was no difference between the two soils in number of fragments found (p = 0.1994; Mann-Whitney U-test) (Fig. 1). This indicates either a higher mortality among adults in the polluted soil than in the reference soil or an enhanced rate of fragmentation and a subsequent high mortality of fragments. More head than tail fragments were recovered from the polluted soil (p = 0.0036; Mann-Whitney U-test) and from the reference soil

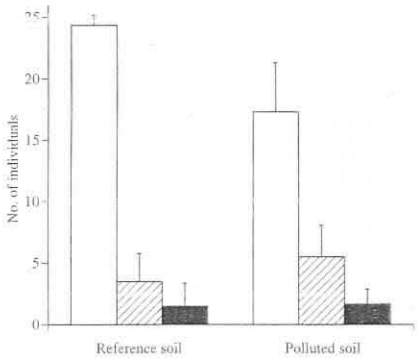

Fig. 1. Number of unchanged adults (>35 segments) and fragments of the enchytraeid *C. sphagnetorum* recovered after two weeks of incubation in the dispersal experiment. 30 worms were inoculated at the start of the experiment. Two mor soils were used: reference and polluted soil (130 and 1670 mg Cu + Zn kg^{-1} soil dry mass; see Table 1). Bars denote adults (white), head fragments (hatched), and tail fragments (black). Mean (n = 6) and SD are given

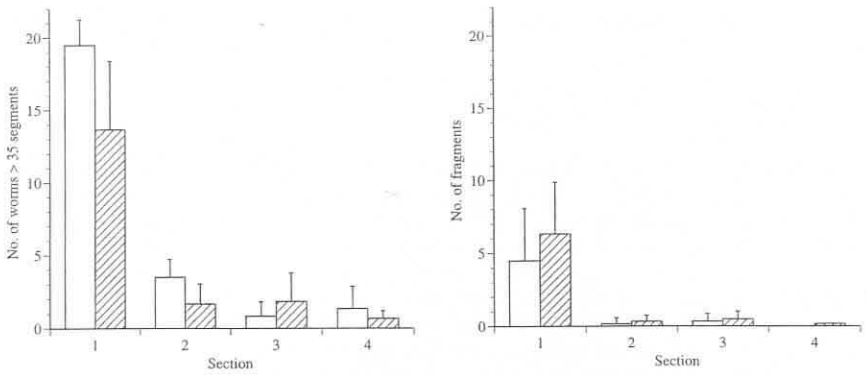

Fig. 2. The distribution of unchanged adults (A) and fragments (B) of *C. sphagnetorum* over four sections after two weeks of incubation. Dispersal abilities of worms were tested in different soils, viz. reference soil (white bars) and polluted soil (hatched bars) (see, experimental soil 1 and 3 in Table 1), using tubes consisting of four interconnected sections. 30 adult worms were inoculated in section 1 at the start of the experiment. Mean (n = 6) and SD are given

(p = 0.0553; Mann-Whitney U-test). No middle fragments were obtained at all. Head fragments tended to consist of fewer segments in polluted (20.3 ± 8.1) than in reference soil (24.3 ± 8.1) (p = 0.057; Mann-Whitney, U-test) indicating that smaller worms fragmented in polluted than in reference soil or that more fragments were formed.
Indepedent of soil metal concentration, almost 80% of the unchanged worms were still found in the section where they had been inoculated (Fig. 2A). There was neither a difference in the distribution of unchanged worms over the tubes nor a difference in the distribution of fragments between the two soils (Kolmogorov-Smirnov) (Fig. 2B). The data fit between the distribution of unchanged worms and the distribution pattern predicted by Buechner's (1987) dispersal model was closest when the probability to stop in the reference soil was p = 0.77 ± 0.07 and in the polluted soil when p = 0.76 ± 0.18. This indicates a similar dispersal independent of soil metal concentration.
Avoidance. On average, 67% of the unchanged worms remained in the ring where they had been inoculated four weeks earlier (Fig. 3A). The higher the difference of metal concentration between the two joined rings, the lower the number of unchanged worms

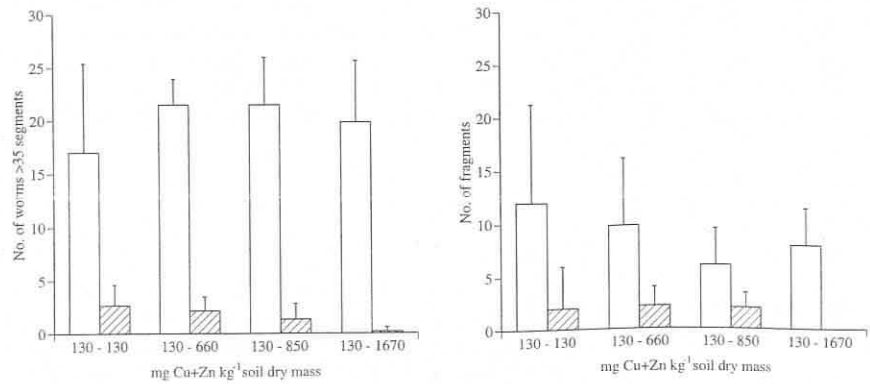

Fig. 3. The distribution of unchanged adult worms (A) and fragments (B) at the end of the avoidance experiment in which two sections with mor soils of the experimental series (Table 1) were joined. At the start of the experiment 30 adults were added to the section with reference soil. The numbers of staying (white) and moving (hatched) adults and fragments were determined after four weeks of incubation. Mean (n = 6) and SD are given

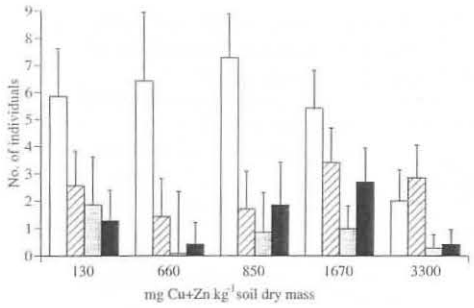

Fig. 4. Number of *C. sphagnetorum* recovered after two weeks of incubation in six experimental soils (see Table 1). Ten adult worms were added to each soil at the start of the experiment. Mean (n = 7) and SD of unchanged worms (white) and head (hatched), middle (stippled) and tail (black) fragments are given

found in the non-inoculated ring ($p = 0.0169$; Kruskal-Wallis) (Fig. 3A). Of the few fragments found, the numbers in the non-inoculated ring did not differ significantly between the treatments ($p = 0.07$; Kruskal-Wallis), though no fragments were found in the soil of the highest metal concentration (1670 mg Cu + Zn kg^{-1} soil dry mass) (Fig. 3B).

Fragmentation and population size. Neither unchanged adults nor fragments were extracted from the most polluted soil (6830 mg Cu + Zn kg^{-1} soil dry mass) after two weeks of incubation (Fig. 4). More unchanged adults per sample were recovered from soils of a metal content ranging between 130 and 1670 mg Cu + Zn kg^{-1} soil dry mass than from soils of a metal content of 3300 mg Cu + Zn kg^{-1} soil dry mass ($p = 0.0001$; Mann-Whitney). The largest fragmentation rate was observed in worms reared in the soil of a metal content of 3300 mg Cu + Zn kg^{-1} soil dry mass and this population differed significantly from those reared in the other soils ($p = 0.0001$; ANOVA) (Table 2). More fragments than unchanged adults were extracted from the soils containing 1670 and 3300 mg Cu + Zn kg^{-1} soil dry mass than from soils of lower metal contents ($p < 0.05$; Mann-Whitney).

Table 2. Rate of fragmentation (n = 7). Mean and SD are given

metal concentrations (mg Cu + Zn kg^{-1} dry mass)	rate of fragmentation (no. fragments day^{-1})
130	0.021 ± 0.014
660	0.019 ± 0.023
850	0.015 ± 0.013
1670	0.028 ± 0.010
3300	0.052 ± 0.013
6830	–

Generally, minimum population size decreased with increasing soil metal concentration in all three experiments (Table 3). In the fragmentation experiment population size did not differ between the soils of 130 to 1670 mg Cu + Zn kg^{-1} soil dry mass but the ratio of unchanged adults to fragments decreased (Fig. 4).

Hyphal lengths. The total length of the active mycelium in the reference soil was significantly greater in reference than in heavily polluted soil ($p = 0.0036$; ANOVA). No significant difference in hyphal lengths was, however, found between reference and mixed soils (130 and 1670 mg Cu + Zn kg^{-1} soil dry mass, respectively) (Table 1).

Metal uptake. Sampled *C. sphagnetorum* (unchanged and fragments) from reference soil contained on average 7.21 ± 7.22 mg Cu kg^{-1} worm tissue (dry mass), which was significantly less than the concentration in the body tissues of the worms sampled from other soils of the series ($p = 0.0377$; ANOVA). The Cu concentration of worms reared in soils of higher metal content did not significantly differ; they had on average a concentration

Table 3. Minimum population size, the total number of unchanged worms and all fragments, obtained after two and four weeks of incubation. Significance values of the difference between treatments are given below the final population size of the experiment. Mean and SD are given

experiment	incubation time (weeks)	metal concentration (mg kg^{-1} dry mass)	population size	
			initial	final
dispersal (n = 6)	2	130	30	29.5 ± 3.7
	2	1670	30	25.2 ± 1.9
			(p = 0.0301 Mann-Whitney U-test)	
avoidance (n = 6)	4	130–130	30	35.7 ± 4.3
	4	130–660	30	35.3 ± 4.8
	4	130–850	30	31.2 ± 2.6
	4	130 – 1670	30	27.8 ± 5.8
			(p = 0.0259 Kruskal-Wallis)	
fragmentation (n = 7)	2	130	10	11.1 ± 1.7
	2	660	10	10.6 ± 3.1
	2	850	10	12.0 ± 2.7
	2	1670	10	12.4 ± 1.7
	2	3300	10	5.14 ± 3.0
			(p = 0.0022 Kruskal-Wallis)	

of 21.39 ± 11.09 mg Cu kg^{-1} tissue dry mass (Fig. 5A). Zinc content of the worm body was positively correlated to the zinc concentration of the soil resulting in a significant difference between the treatments (p = 0.0001; ANOVA) (Fig. 5B).

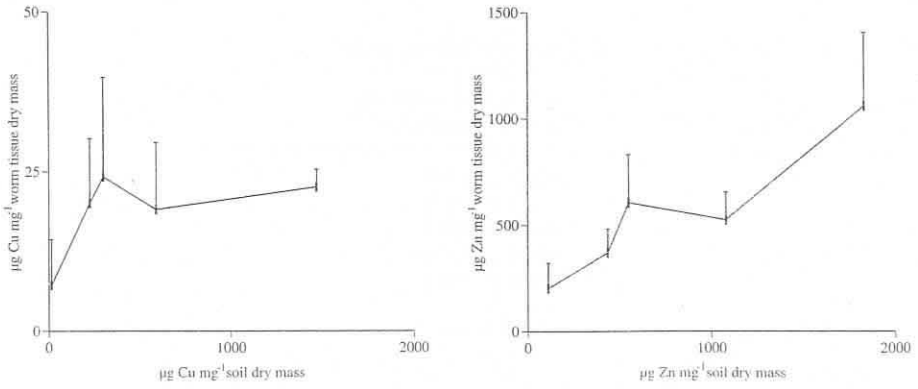

Fig. 5. Copper (A) and zinc (B) contents (µg mg^{-1} dry mass) of worm tissues after two weeks of incubation in soils of different metal concentration (see Table 1). Metals were analysed from batches of 1–4 worms per batch. Mean (n = 7–11) and SD are given

Discussion

The maintenance of small enchytraeid populations at heavily Cu + Zn polluted field sites (Bengtsson & Rundgren 1982) implies the existence of mechanisms which make it possible for individuals to cope with the direct and indirect effects of the metals. This is emphasized by the findings that there was a high mortality of *Cognettia* in experiments with homogenized soil where no specimens survived in the most polluted one and 50% died in soils of half this concentration of copper and zinc. At least three explanations could be given to explain

the persistence of enchytraeid populations at the polluted field sites: continuous immigration, adaptation, and spatial heterogeneity.

5% of the *C. sphagnetorum* population had moved 6 cm in two weeks which implies that individual worms may be able to travel approximately 0.8 – 1.2 m yr^{-1} (assuming a straight forward movement and a temperature-dependent activity period of 6 – 9 months yr^{-1}). Their limited digging ability (Didden 1990) makes it probable that the movement of enchytraeids is facilitated by pores made by roots or by the few earthworms and large arthropods present in coniferous forest soils, allowing them to travel a little further than calculated on the basis of our experiments. Even so the rate of dispersal is low and it seems unlikely that populations will be maintained at polluted sites by a continuous immigration over several hundred meters. Though there is no evidence, vectors, such as man, moose, birds, etc., may carry an unknown number of enchytraeids (either adults, eggs or fragments). It seems more likely, though, that adaptations and/or an ability to find less polluted patches and avoid polluted ones within a heterogeneous site determine the capacity of a *C. sphagnetorum* population to survive.

Although focus was not on genetic adaptation in the present study, it is known that some Collembola species in the Gusum area have adapted genetically in response to high soil metal concentrations (Tranvik et al. 1993, 1994) thus maintaining populations in polluted areas. On the other hand, and maybe more relevant to enchytraeids, the earthworm *Dendrobaena octaedra* (Savigny) did not show any clear adaptive response although present at low densities in polluted soil (Bengtsson et al. 1992).

The scale of the mosaic of more or less polluted patches is thus probably crucial for the maintenance of a population. Favourable patches must be within a range of a few centimeters as worm mortality increases in polluted soil while dispersal rate is just as low as in non-polluted soil. An ability to avoid less favourable soil allows the worms to follow passages of relatively good quality. When reaching a new patch the fragmenting mode of reproduction of *C. sphagnetorum* allows a rapid colonization.

Although copper and zinc are essential metals for most terrestrial invertebrates, high concentrations are toxic (Hopkin 1989). Similar to previous findings (Rüther & Greven 1990) our study reveals a linear uptake of zinc over time, whereas copper accumulates to a certain body concentration (20 $\mu g\ mg^{-1}$ tissue dry mass), which does not further increase with exposure time. The most probable explanation is that specimens with body burdens above this concentration did not survive as mortality also increased with metal concentration of the soil. A shift in feeding habits or an effective excretion of copper are possible alternative mechanisms facilitating survival.

The increased rate of fragmentation at metal concentrations above 850 mg Cu + Zn kg^{-1} soil dry mass could be a direct physiological response to stress or a strategy of early reproduction when conditions are deteriorating. A similar reaction has been observed in response to desiccation (Latter & Howson 1978). Lumbricid earthworms are able to accumulate metals in waste nodules in the posterior coelomic sacs, which led Anderson & Laursen (1982) to suggest that an autotomy encompassing the last segements constitutes a detoxifying mechanism. Whether a similar mechanism associated with fragmentation exists in *C. sphagnetorum* further studies may reveal.

A growing concern of soil contamination and soil protection has led to a demand for new and improved tests evaluating the impact of pollutants and remediation programs on terrestrial ecosystems based on a better understanding of mechanisms and processes in the soil. The present study reflects that need and also aims at presenting a starting-point for the construction of some new tests for risk assessment based on population parameters other than mortality. A number of reasons make the enchytraeid *C. sphagnetorum* a candidate for being included in a test battery based on responses of soil invertebrate species. The species is abundant and plays an important role in decomposition of organic matter and turnover of plant nutrients in acid coniferous forest soils (Persson & Lohm 1977; Römbke 1991). Further, inhabiting the uppermost centimeters of the soil layer (Springett 1963; Bengtsson & Rundgren 1982) they will be affected by silvicultural (Huhta et al. 1967) and

agricultural (O'Connor 1967; Wallwork 1976) practices and by pollutants accumulating in the top layers of the soil, such as metals (Bengtsson & Rundgren 1982), fertilizers (Lohm et al. 1977), and acidifying substances (Lundkvist 1977; Bååth et al. 1980). In addition, *C. sphagnetorum* is possible to handle in laboratory experiments where impacts on biological variables can be monitored. In the present study we were able to show that fragmentation rate and population growth are affected by metals while further studies have to be carried out if a test should be based on dispersal ability.

Acknowledgements

The work was supported by grants from the Swedish Environmental Protection Agency and the Swedish Natural Science Research Council (in cooperation with the EU Programme Environment 1990–1994, contract no. ERB-EV5V-CT92-0218).

References

Abrahamsen, G. (1971) The influence of temperature and soil moisture on the population density of *Cognettia sphagnetorum* (Oligochaeta: Enchytraeidae) in cultures with homogenized raw humus. Pedobiologia **11**, 417–424.

Abrahamsen, G. (1972) Ecological study of Enchytraeidae (Oligochaeta) in Norwegian coniferous forest soils. Pedobiologia **12**, 26–82.

Andersen, C., Laursen, J. (1982) Distribution of heavy metals in *Lumbricus terrestris*, *Aporrectodea longa* and *A. rosea* measured by atomic absorption and X-ray fluorescence spectrometry. Pedobiologia **24**, 347–356.

Arnebrant, K., Bååth, E., Nordgren, A. (1987) Copper tolerance of microfungi isolated from polluted and unpolluted forest soil. Mycologia **79**, 890–895.

Bååth, E., Berg, B., Lohm, U., Lundgren, B., Lundkvist, H., Rosswall, T., Söderström, B., Wirén, A. (1980) Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia **20**, 85–100.

Bengtsson, G., Gunnarsson, T. (1984) A micromethod for the determination of metal ions in biological tissues by furnace atomic absorption spectrophotometry. Microchem. J. **29**, 282–287.

Bengtsson, G., Rundgren, S. (1982) Population density and species number of enchytraeids in coniferous forest polluted by a brass mill. Pedobiologia **24**, 211–218.

Bengtsson, G., Rundgren, S. (1988) The Gusum case: a brass mill and the distribution of soil Collembola. Can. J. Zool. **66**, 1518–1526.

Bengtsson, G., Berden, M., Rundgren, S. (1988a) Influence of soil animals and metals in decomposition processes: A microcosm experiment. J. Environ. Qual. **17**, 113–119.

Bengtsson, G., Ek, H., Rundgren, S. (1992) Evolutionary response of earthworms to long-term metal exposure. Oikos **63**, 289–297.

Bengtsson, G., Erlandsson, A., Rundgren, S. (1988b) Fungal odour attracts soil Collembola. Soil Biol. Biochem. **20**, 25–30.

Bengtsson, G., Hedlund, K., Rundgren, S. (1991) Selective odor perception in the soil Collembola *Onychiurus armatus*. J. Chem. Ecol. **17**, 2113–2125.

Brockmeyer, V., Schmid, R., Westheide, W. (1990) Quantitative investigations of the food of two terrestrial enchytraeid species (Oligochaeta). Pedobiologia **34**, 151–156.

Buechner, M. (1987) A geometric model of vertebrate dispersal: tests and implications. Ecology **68**, 310–318.

van Capelleveen, H. E., van Straalen, N. M., van den Berg, M., van Wachem, E. (1986) Avoidance as a mechanism of tolerance for lead in terrestrial arthropods. Proc. 3rd European Congress Entomology, pp. 251–255.

Cavallaro, N., McBride, M. B. (1980) Activities of Cu^{2+} and Cd^{2+} in soil solutions as affected by pH. Soil Sci. Soc. Am. J. **44**, 729–732.

Christensen, B. (1959) Asexual reproduction in the Enchytraeidae (Olig.). Nature **184**, 1159–1160.

Coleman, D. C., Ingham, R. E., McClellan, J. F., Trofymow, J. A. (1984) Soil nutrient transformations in the rhizosphere via animal-microbial interactions. In: Anderson, J. M., Rayner, A. D. M.,

Walton, D. W. H. (eds) Invertebrate-microbial interactions. Cambridge University Press, Cambridge. pp. 35–58.
Dash, M. C., Cragg, J. B. (1972) Ecology of Enchytraeidae (Oligochaeta) in Canadian Rocky Mountain soils. Pedobiologia **12**, 323–335.
Didden, W. A. M. (1990) Involvement of Enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. Biol. Fertil. Soils **9**, 152–158.
Erman, D. C. (1973) Invertebrate movements and some diel and seasonal changes in a Sierra Nevada peatland. Oikos **24**, 85–93.
Frankland, J. C. (1992) Mechanisms in fungal succession. In: Carroll, G. C., Wicklow, D., T. (eds) The fungal community, its organization and role in the ecosystem. Marcel Dekker, Inc., New York. pp. 383–401.
Hopkin, S. P. (1989) Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Science Publishers Ltd, London.
Huhta, V. (1984) Response of *Cognettia sphagnetorum* (Enchytraeidae) to manipulation of pH and nutrient status in coniferous forest soil. Pedobiologia **27**, 245–260.
Huhta, V., Karppinen, E., Nurminen, M., Valpas, A. (1967) Effect of silvicultural practices upon arthropod, annelid and nematod populations in coniferous forest soil. Ann. Zool. Fenn. **4**, 7–143.
Kareiva, P. (1982) Experimental and mathematical analyses of herbivore movement; quantifying the influence of plant spacing and quality on foraging discrimination. Ecol. Monographs, **52**, 261–282.
Kareiva, P. (1983) Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement. In: Denno, R. F., McClure, M. S. (eds) Variable plants and herbivores in natural and managed systems: Academic Press, New York. pp. 259–289.
Lagerwerff, J. V., Specht, A. W. (1970) Contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc. Environ. Sci. Technol. **4**, 583–588.
Latter, P. M., Howson, G. (1978) Studies on the microfauna of blanket bog with particular reference to Enchytraeidae. J. Anim. Ecol. **47**, 425–448.
Lohm, U., Lundkvist, H., Persson, T., Wirén, A. (1977) Effects of nitrogen fertilisation on the abundance of enchytraeids and microarthropods in Scots pine forests. Studia Forestalia Suecia **40**, 1–23.
Lundkvist, H. (1977) Effects of artificial acidification on the abundance of Enchytraeidae in Scots pine forest in northern Sweden. In: Lohm U. and T. Persson (eds) Soil organisms as components of ecosystems. Ecol. Bull. **25**, 570–573.
Lundkvist, H. (1982) Population dynamics of *Cognettia sphagnetorum* (Enchytraeidae) in a Scots pine forest soil in Central Sweden. Pedobiologia **23**, 21–41.
Makulec, G. (1983) Enchytraeidae (Oligochaeta) of forest ecosystems: I. Density, biomass and production. Ekologia Polska **31** A, 9–56.
Maynard Smith, J. (1971) What use is sex? J. Theor. Biol. **30**, 319–335.
Nielsen, C. O. (1955) Studies on Enchytraeidae. 5. Factors causing seasonal fluctuations in numbers. Oikos **6**, 153–169.
Nordgren, A., Bååth, E., Söderström, B. (1983) Microfungi and microbial activity along a heavy metal gradient. Appl. Environ. Microbiol. **45**, 1829–1837.
O'Connor, F. B. (1967) The Enchytraeidae. In: Burges, A., Raw, F. (eds) Soil biology. Academic Press, London. pp. 213–257.
Peachey, J. E. (1963) Studies on the Enchytraeidae (Oligochaeta) of moorland soil. Pedobiologia **2**, 81–95.
Persson, T., Lohm, U. (1977) Energetical significance of the annelids and arthropods in a Swedish grassland soil. Ecol. Bull. **23**. Swedish Natural Science Research Council, Stockholm.
Rüther, U., Greven, H. (1990) The effect of heavy metals in enchytraeids. I. Uptake from an artifical substrate and influence on food preference. Acta Biol. Benrodis **2**, 125–131.
Römbke, J. (1991) Estimates of the Enchytraeidae (Oligochaeta, Annelida) contribution to energy flow in the soil system of an acid beech wood forest. Biol. Fertil. Soils **11**, 255–260.
Söderström, B. E. (1977) Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. Soil Biol. Biochem. **9**, 59–63.
Sokal, R. R., Rohlf, F. J. (1980) Biometry. W. H. Freeman and Company, New York.
Springett, J. A. (1963) The distribution of three species of Enchytraeidae in different soil. In: Doeksen, J., van der Drift, J. (eds) Soil organisms. North-Holland Publ. Co., Amsterdam, pp. 414–418.
Springett, J. A. (1970) The distribution and life histories of some moorland Enchytraeidae (Oligochaeta). J. Anim. Ecol. **39**, 725–737.
Springett, J. A., Brittain, J. E., Springett, B. P. (1970) Vertical movement of Enchytraeidae (Oligochaeta) in moorland soils. Oikos **21**, 16–21.

Standen, V. (1973) The production and respiration of an enchytraeid population in blanket bog. J. Anim. Ecol. **42**, 219–245.
Swift, M. J., Boddy, L. (1984) Animal-microbial interactions in wood decomposition. In: Anderson, J. M., Rayner, A. D. M., Walton, D. W. H. (eds) Invertebrate-microbial interactions. Cambridge University Press, Cambridge. pp. 89–131.
Tranvik, L., Bengtsson, G., Rundgren, S. (1993) Relative abundance and resistance traits of two Collembola species under metal stress. J. Appl. Ecol. **30**, 43–52.
Tranvik, L., Sjögren, M., Bengtsson, G. (1994) Allozyme polymorphism and protein profile in *Orchesella bifasciata* (Collembola): Indicative of extended metal pollution? Biochem. Syst. Ecol. **22**, 13–23.
Turchin, P. (1991) Translating foraging movements in heterogeneous environments into the spatial distribution of foragers. Ecology **72**, 1253–1266.
Tyler, G. (1974) Heavy metal pollution and soil enzymatic activity. Plant and Soil **41**, 303–311.
Tyler, G. (1978) Leaching rates of heavy metal ions in forest soil. Water, Air, and Soil Pollution **9**, 137–148.
Usher, M. B. (1976) Aggregation responses of soil arthropods in relation to the soil environment. In: Andersson, J. M., Macfadyen, A. (eds) The role of terrestrial and aquatic organsims in decomposition processes. Blackwell Scientific Publications, Oxford. pp. 61–94.
Wallwork, J. A. (1976) The distribution and diversity of soil fauna. Academic Press Inc. (London) Ltd., London.